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The effects of soil temperature on urea hydrolysis, nitrification, and plant utilization

Wayne B. Fisher

To the Graduate Council:

I am submitting herewith a thesis written by Wayne B. Fisher entitled "The effects of soil temperature on urea hydrolysis, nitrification, and plant utilization." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

W. L. Parks, Major Professor

We have read this thesis and recommend its acceptance:

L. N. Skold, L. F. Seats

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 8, 1957

To the Graduate Council:

I am submitting herewith a thesis written by Wayne B. Fisher, Jr. entitled "The Effects of Soil Temperature on Urea Hydrolysis, Nitrification, and Plant Utilization." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

W. L. Parks

We have read this thesis
and recommend its acceptance:

L N Skold

Lloyd F. Seatz

Accepted for the Council:

Charles P White for

Dean of the Graduate School

THE EFFECTS OF SOIL TEMPERATURE ON UREA HYDROLYSIS, NITRIFICATION, AND PLANT UTILIZATION

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

Wayne B. Fisher, Jr.

March 1957

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Wayne B. Fisher, Jr.

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CHAPTER I

INTRODUCTION

Urea is being produced in large amounts for use in plastics, feeds and fertilizers. Although it has been used as a fertilizer for many years, it has not been until recent years that adequate supplies have been available for extensive use as a fertilizer. It was the purpose of this investigation to study some of the factors that influence the availability of urea nitrogen to plants. The two main objectives of this study were: (1) to determine the rate of urea hydrolysis and subsequent nitrification to nitrate nitrogen at several controlled soil temperatures, and (2) to determine the yield and chemical composition of ryegrass forage grown under controlled conditions with different rates of nitrogen fertilization from urea and ammonium nitrate.

CHAPTER II

REVIEW OF LITERATURE

Urea was discovered in 1790 by Roule as a compound occurring in the urine of mammals, especially in that of the flesh eaters. Since then urea has been found in small quantities in blood, muscle tissue, saliva and other fluids of animals. Also, minute quantities have been found in the leaves of spinach and carrots and in seedlings of various other plants (17). As expected, fresh farm manure contains appreciable quantities of urea. The decomposition of this compound is mainly responsible for the odor of ammonia associated with fresh manure piles.

Pasteur (25) in 1860 was the first to recognize that the transformation of urea to ammonia is brought about by living organisms. It was later discovered that organisms capable of hydrolyzing urea are found in most families of bacteria, actinomycetes and fungi (25). However, it was considered by Sumner and Somers (23) that the bacteria employ urease to produce ammonia for their needs. Urease, an enzyme, was isolated in crystalline form from jack bean meal in May 1926 by Sumner (25) and was the first enzyme obtained in a pure condition. Conrad (6) has reported that urease activity in the soil may vary with the cropping system, and the higher the organic matter content, the greater the urease-like activities. Consequently, the surface soil is higher than the subsoil in these activities.

Conrad and Adams (8) suggested that the hydrolysis of urea should be considered catalytic in nature instead of completely microbial. After sterilization of soils with toluene it was found that urea was hydrolyzed almost as rapidly as if the soils had not been treated with toluene (7).

The rate of urea hydrolysis was studied by Laidler and Hoard (15) in buffered solutions at various pH values and at different temperatures. It was reported that the maximum rate of urea hydrolysis was at a pH of 6.2 and a temperature of 30°C. Gibson (11) reported that strongly acid peat samples of pH 3.1 to 3.3 hydrolyzed urea rapidly at 20 to 23°C. Conrad (7) quoted Rubentschik as experimenting with two organisms that were capable of decomposing urea below 0°C. Conrad (7), also, reported that urea was hydrolyzed at 2°C but not at 90°C.

The ammonia formed by urea hydrolysis becomes the raw material for the process of nitrate formation (6). Since nitrification is, by definition, the oxidation of ammonia to nitrate nitrogen (24), the speed of nitrification is partly dependent upon the quantity of available ammonia.

Nitrification is dependent upon bacteria. There are two groups of these bacteria. One group, Nitrosomonas or Nitrosococcus, transforms ammonia to nitrate nitrogen and the other group, nictrobacter, converts the nitrite to nitrate nitrogen (26). These reactions depend upon favorable soil reaction, aeration, amount of organic matter, temperature, moisture, and concentration of inorganic substances in the soil (10).

Morgan (18) reported that 2.62 pounds of calcium carbonate were required to neutralize the soil reaction produced by each pound of nitrogen added to the soil as urea; whereas, Pierre (20) reported that only thirty-six pounds of calcium carbonate per unit of nitrogen were required.

A number of studies of crop response to different nitrogen fertilizers have been conducted, and many of these have been summarized by Andrews (1). Generally there have been no great differences in sources of nitrogen.

There was little information published on the composition of ryegrass grown at various temperatures until 1949 when Sullivan and Sprague (22) reported the results of a study dealing with the effects of temperature on the chemical composition of perennial ryegrass. The temperatures were allowed to vary by 10°F; the day temperatures were 10°F above the night temperatures. The light intensity at which these plants grew after entering the growth chambers was 525 footcandles. They found new top growth to be most rapid at 60 to 70°F and least at 80 to 90°F. It was concluded that high temperatures adversely affected ryegrass by rapid dissipation of reserve carbohydrates, slowing down the production of new leaf growth and in general inhibiting recovery from clipping. At the highest temperatures studied, the soluble nitrogen increased in the tops and stubble of the ryegrass and this increase continued for some time. Associated with this nitrogen increase, was a decrease in carbohydrates. Forty days after clipping, the carbohydrate supply was almost exhausted. They reported that the calcium content of the roots and stubble were slightly higher at the highest temperature.

Effects of temperature on growth of several other grasses have been reported. According to Harrison (13), growth of Kentucky bluegrass was more nearly normal at 60°F than at other temperatures studied. At 80°F top growth was rapid but no new roots were initiated and shoots from rhizomes appeared above ground only under low nitrogen conditions. The bluegrass grew little at 100°F and died after about six weeks at that temperature. Lovvorn (16) found that yields obtained by frequent cuttings were higher at 65°F than at 85°F for Dallisgrass, carpetgrass, Bermudagrass and Kentucky bluegrass. Brown (4) reported that the protein content of Kentucky bluegrass, Canada Bluegrass and orchardgrass was at a minimum at 60°F-70°F and was greater at both lower and higher temperatures. Bermudagrass growth was greatest at 100°F but the protein content was at a minimum at temperatures between 80°F to 90°F. This is somewhat higher than for the other grasses studied.

CHAPTER III

METHODS AND PROCEDURES

Three growth chambers were constructed for use in this study and set at temperatures of 10°C, 20°C and 30°C. These temperatures were thermostatically controlled and maintained the desired temperature within an accuracy of ± 1°C. The internal volume of each chamber was 100 cu. ft. and a shelf to support 15 pots was suspended in the middle of each chamber. Twenty fluorescent lights, using cool white and red tubes at a ratio of 9:1 were used as the light source. This gave light intensities on the ryegrass seedlings of 805, 1119 and 1222 footcandles for the 10, 20 and 30°C temperatures respectively. Similar relations between temperature and light intensity have been reported by Went (27).

Hermitage silt loam soil was used in all phases of this study. It is a highly productive soil and the samples used were purposely selected because of their low ammonia and nitrate nitrogen content. The soil was kept moist at all times during transfer from the field to the growth chambers so as to maintain the native soil flora.

A. Hydrolysis and Nitrification.

Samples of the soil were treated with urea at the rate of 100 and 200 pounds of nitrogen per 2,000,000 pounds of soil. Sub-samples, including a no-treatment check, were pre-weighed, transferred to flasks or beakers and placed in the temperature controlled chambers. The soil was maintained at 15 to 20 per cent moisture content throughout this experiment.

Duplicate samples were analyzed at the end of the first, second, third and fifth weeks for ammonia, nitrate and pH. Nitrate nitrogen was determined by the phenoldisulphonic acid method as described by Jackson (14). Ammonia nitrogen was extracted with sodium chloride and determined by the distillation method described by Peech et al. (19). Urea was determined by difference. The pH of the soil was determined using the Beckman model "G" pH meter.

B. Ryegrass Forage.

Ryegrass was grown under controlled conditions at different rates of nitrogen fertilization. Forty-five samples, containing the equivalent of 2485 grams of oven dry (110°C) soil, were placed in gallon cans lined with plastic bags, and were seeded to annual ryegrass (Lolium multiflorum) October 19, 1956. These were placed in the greenhouse until the ryegrass was about three inches high. Urea or ammonium nitrate solutions were then added to these containers November 13, 1956, at rates equivalent to 50 and 100 pounds of nitrogen per acre and these treated containers along with checks were randomly distributed in the growth chambers. The same randomization was used in each growth chamber and there were three replications of each nitrogen treatment in each growth chamber.

Alternating periods of sixteen hours of light and eight of darkness were maintained throughout the course of the experiment. The light period was from 6 PM until 10 AM so as not to place excessive load on the cooling systems of the growth chambers. A small heat source was turned on in each growth chamber during the dark period. A ten inch fan was in operation at all times to insure good air circulation and to keep the growth chamber free from cold spots.

The plants were harvested fifty-seven days after the addition of the nitrogen. At time of harvest the plants grown at 20°C and 30°C were flowering, whereas those at 10°C were not. Fresh and oven dry (70°C) weights of the forage per container were obtained. The dried tissue was ground on a Wiley mill, mixed thoroughly, and stored in bottles for analysis. The nitrogen content of the ryegrass tissue was determined by the Gunning Kjeldahl method as described by the Association of Official Agricultural Chemists (2). A modification of the wet oxidation procedure of Gieseking, Snider and Getz (12) was used for ashing all samples. The potassium, calcium, and magnesium content of the tissue was determined by a Beckman "DU" flame spectrophotometer equipped with a photomultiplier attachment. The molybdenum blue method as described by Bray (3) was used to determine the phosphorus content of the tissue on a Beckman "B" spectrophotometer.

Detailed procedures are found in the Appendix, pages 36 through 41.

CHAPTER IV

RESULTS AND DISCUSSION

Urea Conversion

The results of urea hydrolysis to ammonia, its subsequent conversion to nitrate nitrogen and the soil reaction associated with this process at 10°C, 20°C and 30°C temperature are presented in figure I and table I. The ammonia and nitrate nitrogen values are the average of duplicate sample determinations. The values represent the differences between a check and treated sample. The amount of urea was determined by difference.

Maximum ammonia concentrations occurred at the end of the first week and decreased rapidly thereafter at all temperatures except at 10°C. At 30°C, approximately 84 per cent of the urea was hydrolyzed by the end of the first week. At 20°C, approximately 76 per cent of the 100-pound per acre rate was hydrolyzed and 38 per cent of the 200-pound per acre rate at the end of the first week. These figures represent close to the same number of pounds of nitrogen hydrolyzed. At 10°C, only 53 per cent of the 100-pound per acre rate and 28 per cent of the 200-pound per acre rate were hydrolyzed by the end of the first week, and about the same number of pounds of urea nitrogen was hydrolyzed at each rate. These data clearly indicate the influence of temperature upon the rate of urea hydrolysis. This is further evidenced by the fact that 84 per cent of both rates of nitrogen were hydrolyzed at 30°C by the end of the first

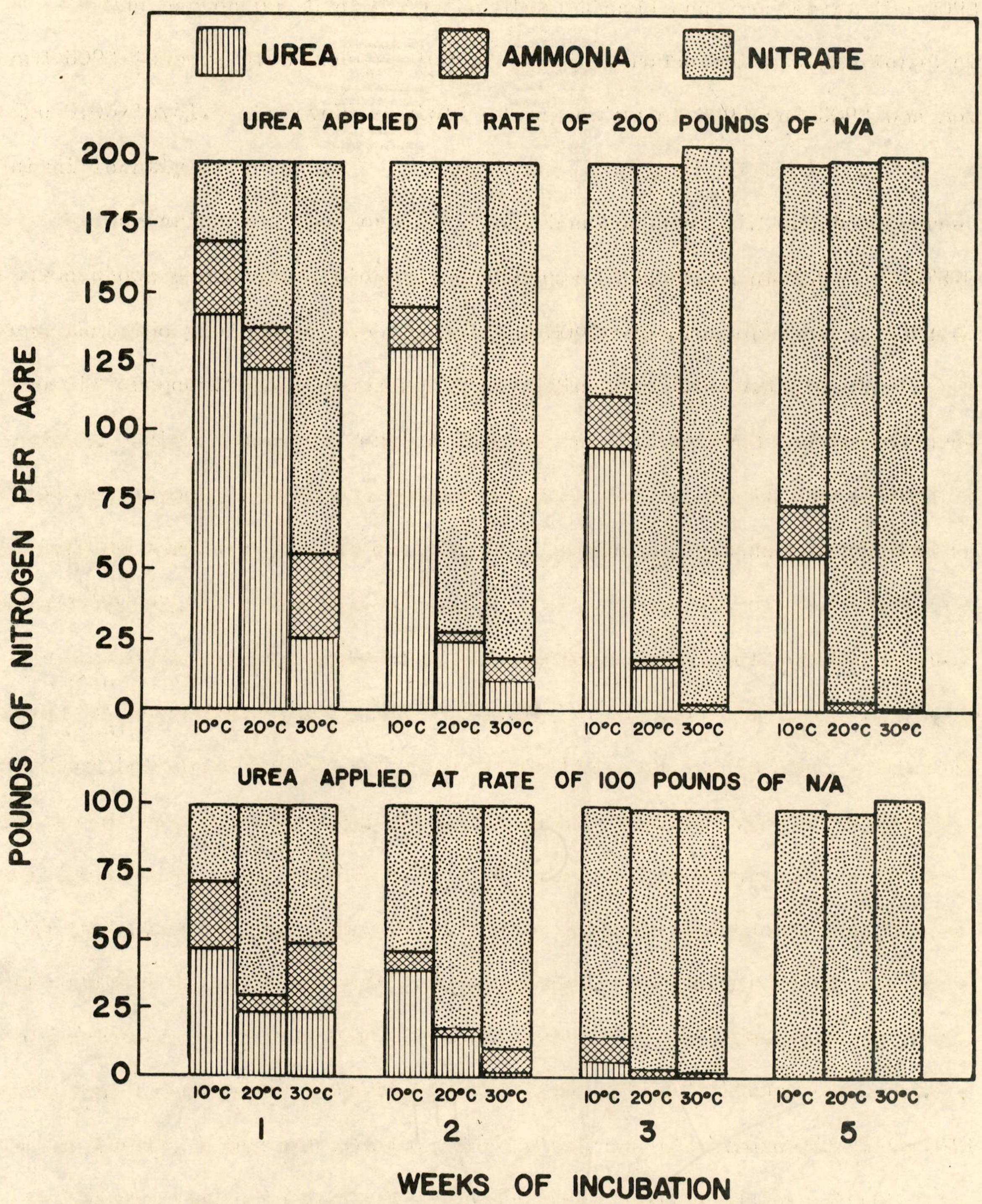

FIGURE 1. — AMOUNT OF UREA, AMMONIA, AND NITRATE NITROGEN IN THE SOIL AT WEEKLY INTERVALS FOLLOWING APPLICATIONS OF UREA AND INCUBATION AT VARIOUS TEMPERATURES.

week, which represents a wide difference in pounds of nitrogen hydrolyzed per acre.

At the 20°C and 30°C temperatures over 85 per cent of the urea had been hydrolyzed by the end of the second week. Hydrolysis at the 100- and 200-pound rate at 10°C was again similar by the end of the second week with 61 per cent of the 100-pound rate being hydrolyzed and 34 per cent of the 200-pound rate being hydrolyzed. There was a difference of seven pounds of total nitrogen hydrolyzed at the end of the second week, which indicates that the amount of urea present had little effect upon the rate of urea hydrolysis at 10°C. By the end of the third week, over 80 per cent of the urea had been hydrolyzed at the 100-pound per acre rate at 10oC. At the 200-pound rate 52 per cent of the urea had hydrolyzed by the end of the third week and 72 per cent by the end of the fifth week. Higher temperatures produced an increase in the rate of hydrolysis in all cases. The effect of rate of urea fertilization on the amount of urea hydrolyzed was small at 10°C, intermediate at 20°C and was rather pronounced at 30°C.

The 30°C temperature at the 200-pound per acre rate of nitrogen had the most rapid nitrification with 72 per cent, 90 per cent, and 100 per cent being converted to nitrate at the end of the first, second and third weeks respectively. At the 100-pound per acre rate, 50 per cent, 89 per cent, and 100 per cent of the nitrogen was converted at the end of the first, second and third weeks respectively.

At the 20°C temperature, 30 per cent, 86 per cent, and 90 per cent of the 200 pounds of nitrogen per acre rate had been converted to nitrate at the end of first, second and third weeks respectively, and at the 100-pound per acre rate, 70 per cent, 97 per cent and 100 per cent nitrification had occurred at the end of the first, second and third weeks, respectively.

The 10°C temperature had the slowest rate of nitrification and the amount nitrified varied little with the rate of fertilization. There were 29 pounds of nitrate nitrogen present at the 100-pound per acre rate as compared with 28 pounds at the 200-pound per acre rate by the end of the first week. By the end of the second week, the 100-pound per acre rate had 55 and the 200-pound per acre rate had 53 pounds of nitrate nitrogen accumulation. The same situation existed at the end of the third week with approximately 85 pounds of nitrate nitrogen accumulated for both rates of fertilization. Nitrification was complete at the end of the fifth week for the 100-pound per acre rate, but there remained approximately 35 per cent of the nitrogen to be converted to nitrate at the 200 pounds of nitrogen per acre rate at the 10°C temperature.

These data show that there was generally an increase in the rate of urea hydrolysis and subsequent nitrification with increase in temperature. The higher rate of fertilization increased the average amount of hydrolysis and nitrification at all temperatures except at the 10°C. The 10°C temperature had very nearly a constant rate of nitrification, about 25 pounds per week, at both rates of fertilization. This constant rate of nitrification could possibly be related to the rate of supply of ammonia at that temperature.

The soil pH was 6.8 at the beginning of the experiment and changed little during the course of this experiment.

TABLE I

SOIL pH AT WEEKLY INTERVALS FOLLOWING APPLICATION OF UREA AND INCUBATION AT VARIOUS TEMPERATURES

Nitrogen Level	Soil Temp.	Weeks of Incubation				
		0	1	2	3	5
200 lbs. Nitrogen/A.	10°C	6.8	6.8	6.8	6.7	6.5
	20°C	6.8	6.8	6.8	6.7	6.8
	30°C	6.8	6.8	6.6	6.7	6.8
100 lbs. Nitrogen/A.	10°C	6.8	6.7	6.7	6.7	6.5
	20°C	6.8	6.8	6.6	6.7	6.8
	30°C	6.8	6.8	6.6	6.7	6.5
0 lbs. Nitrogen/A.	10°C	6.8	6.6	6.7	6.8	6.6
	20°C	6.8	6.8	6.8	6.8	6.8
	30°C	6.8	6.9	6.8	6.8	6.8

Yield of Ryegrass Forage

Forage yield data are summarized in table II. All data presented in the following tables were subjected to analysis by statistical procedures as outlined by Cochran and Cox (5) or Snedecor (21). Complete data and analyses are found in the Appendix.

Ryegrass yields at the 20°C temperature were significantly higher than those at 10°C or 30°C, and the yield obtained at 30°C was greater than at 10°C, the difference again being highly significant. These results are in agreement with those reported by Sullivan and Sprague (22). They suggested that a possible explanation of the higher yield at 20°C than at 30°C is that the carbohydrates are used almost as rapidly as they are produced. The soil conversion studies indicate that the supply of nitrate nitrogen would not be limiting at 30°C.

A comparison of the yield of the untreated pots with the nitrogen treated pots show a highly significant response to nitrogen.

When comparing the rates of nitrogen the yield at the 100-pound per acre rate was greater than at the 50-pound per acre rate, the difference being highly significant. The greatest yield response of ryegrass forage to nitrogen occurred at 20°C, the increase at this temperature being almost twice that obtained at 30°C. The least response to nitrogen occurred at 10°C.

TABLE II

YIELD OF OVEN DRY RYEGRASS FORAGE IN GRAMS PER POT GROWN AT THREE TEMPERATURES WITH THREE RATES AND TWO SOURCES OF NITROGEN

Pounds N/Acre	Source	Grams Per Pot: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		2.69[a]	4.16	3.38	3.41	3.41
50	Urea	3.01	5.18	3.87	4.02	4.04
	Ammonium Nitrate	3.39	4.99	3.81	4.06	
100	Urea	3.76	5.79	4.47	4.67	4.58
	Ammonium Nitrate	3.26	6.09	4.12	4.49	
Average		3.22	5.24	3.93		
Nitrogen treated average		3.35	5.51	4.07		

L.S.D. (.05) between temperature means - - - - - - - - - 0.38 grams
L.S.D. (.01) between temperature means - - - - - - - - - 0.51 grams
L.S.D. (.05) between rate means - - - - - - - - - - - - - 0.31 grams
L.S.D. (.01) between rate means - - - - - - - - - - - - - 0.42 grams

C.V. for entire experiment - - - - - - - - - - - - - - - 9.92%
C.V. excluding checks - - - - - - - - - - - - - - - - - - 10.34%

[a]Figures represent an average of three replications.

Chemical Composition of the Ryegrass Forage

Data presented are expressed as per cent composition of oven dry (70°C) forage on a weight basis and represent the mean of three replications.

Summaries of nitrogen, potassium, calcium, magnesium and phosphorus composition of the plants are presented in tables III through VII, respectively.

At 10°C and 30°C there was a significantly greater per cent of nitrogen in the forage than at 20°C. This is possibly because of the higher yield and greater growth at 20°C. No significant difference in the per cent nitrogen in the forage produced at 10°C and 30°C was obtained.

At 20°C and 30°C there was a greater percent of potassium and magnesium in the forage than at 10°C, the difference being significant at the 0.05 level. This indicates that the low temperature retarded the uptake of these two nutrients. There was no significant difference between the means of these nutrients at 20°C and 30°C.

When comparing phosphorus in the forage, at different temperatures, the per cent phosphorus at 20°C was greater than at 30°C and the per cent phosphorus at 30°C was greater than at 10°C. The difference, in both cases, was significant at the 0.01 level. It is therefore evident that the phosphorus content of the plant and the yield are directly related.

Although the per cent calcium in the forage increased greatly with an increase in temperature and rates of nitrogen, no significant difference in the per cent calcium at any of the three temperatures was observed. This was perhaps due to a significant interaction between temperature and rates of nitrogen.

TABLE III

PERCENT OF NITROGEN IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Percent Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		1.69[a]	1.27	1.81	1.59	1.59
50	Urea	2.43	1.62	2.43	2.16	2.17
	Ammonium Nitrate	2.03	1.75	2.77	2.18	
100	Urea	2.50	2.04	2.63	2.39	2.35
	Ammonium Nitrate	2.33	1.96	2.64	2.31	
Average		2.20	1.73	2.46		
Nitrogen treated average		2.32	1.84	2.62		

L.S.D. (.05) between temperature means - - - - - - - - - - - - - - 0.28%
L.S.D. (.01) between temperature means - - - - - - - - - - - - - - 0.22%

C.V. for entire experiment - 5.3%
C.V. excluding checks - 5.7%

[a]Figures represent an average of three replications.

TABLE IV

PERCENT OF POTASSIUM IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Percent: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		2.62[a]	2.93	2.97	2.84	2.84
50	Urea	2.53	3.18	3.28	3.00	3.08
	Ammonium Nitrate	3.03	3.32	3.15	3.17	
100	Urea	2.72	3.27	2.97	2.99	3.12
	Ammonium Nitrate	3.02	3.50	3.22	3.25	
Average		2.78	3.24	3.12		
Nitrogen treated average		2.82	3.32	3.16		

L.S.D. (.05) between temperature means - - - - - - - - - - - - 0.34%

C.V. for entire experiment - - - - - - - - - - - - - - - - - - 6.23%
C.V. excluding checks - 6.41%

[a]Figures represent an average of three replications.

TABLE V

PERCENT OF CALCIUM IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Percent: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		0.45[a]	0.53	0.78	0.59	0.59
50	Urea	0.65	0.68	0.92	0.75	0.72
	Ammonium Nitrate	0.53	0.58	0.98	0.70	
100	Urea	0.50	0.72	1.10	0.77	0.82
	Ammonium Nitrate	0.52	0.80	1.25	0.86	
Average		0.53	0.66	1.01		
Nitrogen treated average		0.54	0.70	1.06		

C.V. for entire experiment - 10.19%
C.V. excluding checks - 6.41%

[a] Figures represent an average of three replications.

TABLE VI

PERCENT OF MAGNESIUM IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Percent: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		0.28[a]	0.50	0.61	0.46	0.46
50	Urea	0.27	0.53	0.66	0.49	0.49
	Ammonium Nitrate	0.30	0.43	0.73	0.49	
100	Urea	0.28	0.55	0.71	0.51	0.53
	Ammonium Nitrate	0.28	0.67	0.78	0.55	
Average		0.28	0.54	0.70		
Nitrogen treated average		0.28	0.54	0.72		

L.S.D. (.05) between temperature means - - - - - - - - - - - - 0.24%

C.V. for entire experiment - - - - - - - - - - - - - - - - - - 5.53%
C.V. excluding checks - 4.93%

[a] Figures represent an average of three replications.

TABLE VII

PERCENT OF PHOSPHORUS IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

		Percent				
		Temperature				
Pounds N/Acre	Source	10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		0.14[a]	0.25	0.20	0.20	0.20
50	Urea	0.11	0.20	0.15	0.15	0.16
	Ammonium Nitrate	0.12	0.24	0.16	0.17	
100	Urea	0.14	0.18	0.16	0.16	0.16
	Ammonium Nitrate	0.12	0.18	0.15	0.15	
Average		0.13	0.21	0.16		
Nitrogen treated average		0.12	0.20	0.16		

L.S.D. (.05) between temperature means - - - - - - - - - - - - 0.02%
L.S.D. (.01) between temperature means - - - - - - - - - - - - 0.03%

C.V. for entire experiment - - - - - - - - - - - - - - - - - - 5.78%
C.V. excluding checks - 8.10%

[a] Figures represent an average of three replications.

A comparison of the means of the unfertilized pots with the nitrogen treated pots showed that a highly significant increase in the per cent nitrogen, calcium and phosphorus content of the forage was obtained from the first fifty pounds of nitrogen. No further significant increase in these elements was obtained from additional nitrogen. There was an increase in magnesium per cent in the forage due to nitrogen fertilization; this increase being significant at the 0.05 level. The per cent potassium increased in the forage due to nitrogen treatment but the increase was not significant.

No significant difference in the per cent nitrogen, potassium, calcium, magnesium or phosphorus content of the forage could be attributed to sources of nitrogen.

The yields of nitrogen, potassium, calcium, magnesium and phosphorus in the ryegrass forage are presented in tables VIII through XII respectively. These values were obtained by multiplying the forage yield by the per cent composition of these nutrients and represent a mean of three replications.

The average yield of nitrogen at the 20°C and 30°C temperatures was greater than the yield of nitrogen at the 10°C, the difference being significant at the .01 level. There was no significant difference in the yield of nitrogen between the 20°C and 30°C temperatures.

When comparing the mean yields of potassium in the forage at different temperatures, the yield of potassium at 20°C was greater than that at 10°C and 30°C, and the yield of potassium at 30°C was greater than that at 10°C. The difference in both cases being significant at the 0.05 level.

The magnesium removed in the plant tissue was greater at the 20°C and 30°C temperatures than at 10°C, this difference being significant at the 0.05 level. The average yield of magnesium at 20°C and 30°C was not significantly different.

Comparison of the mean yield of phosphorus at different temperatures shows a greater yield of phosphorus at 20°C than at 10°C or 30°C and that the yield of phosphorus at the 30°C was greater than at 10°C. The difference in both cases being significant at the 0.01 level.

No significant difference in the yield of calcium due to temperature was detected.

Comparisons of the mean yields of nitrogen, potassium, calcium and magnesium of the untreated pots with the average yield of the nitrogen treated pots shows a highly significant increase in yield of these nutrients due to nitrogen fertilization. There was no significant increase in the yield of phosphorus due to the application of nitrogen. This is in agreement with results reported by Domby *et al.* (9).

Considerably more nitrogen was removed in the forage when the plants were fertilized with 100 pounds of nitrogen per acre than with 50 pounds of nitrogen per acre. This increase was significant at the 0.01 level. There was no real difference in the yield of potassium, calcium, magnesium or phosphorus due to the rate of nitrogen applied.

No significant difference due to sources of nitrogen in the yield of nitrogen, potassium, calcium, magnesium or phosphorus was found.

The results of these investigations indicate that urea and ammonium nitrate perform equally well as a nitrogen source for ryegrass.

The rate of urea hydrolysis and subsequent nitrification is influenced by soil temperature. Of the temperatures studied, the fastest rates of hydrolysis and nitrification occurred at 30°C. This corresponds with the findings of Laidler and Hoard (15) and Frederick (10). At 10°C, the rate of urea hydrolysis is relatively slow. This could lead to leaching losses of urea nitrogen applied to the soil at this temperature. Once the urea had hydrolyzed, it would be expected to behave as any ammonia source of nitrogen.

Soil temperature had a greater effect on the yield of ryegrass forage than did the rate of nitrogen fertilization. The highest yield of ryegrass forage was obtained at 20°C but the per cent of nitrogen in the ryegrass was lower at 20°C than at 10 and 30°C. This is similar to results reported by Brown (4). However, the higher yield of forage at 20°C brought the plant removal of nitrogen at this temperature above that at 10°C and almost equal to that at 30°C. This would indicate that at 10°C the ryegrass absorbed the nitrogen rapidly, however, the plants were unable to assimilate it as fast as it was absorbed. The relatively high nitrogen content of the forage at 30°C was probably due to photosynthesis not being able to keep pace with respiration, thereby creating a carbohydrate deficiency (22).

The influence of soil temperature and rate of nitrogen fertilization on calcium content of the forage was quite marked and a significant interaction between these two factors was observed. The per cent of calcium and the yield of calcium increased with increases in both temperature and rate of nitrogen fertilization. A similar relationship was found for magnesium.

The phosphate and potassium content of the forage was directly related to forage yield. The highest content of these nutrients was found at the highest forage yield level and the lowest content at the lowest forage yield. The per cent potassium increased with rates of nitrogen fertilization but the per cent phosphorus decreased with rates of nitrogen fertilization. This is generally the case when potassium-nitrogen and phosphorus-nitrogen are used in combinations.

TABLE VIII

MILLIGRAMS OF NITROGEN PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Milligrams Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		45.4[a]	53.1	61.1	53.2	53.2
50	Urea	73.0	85.3	93.5	83.9	85.4
	Ammonium Nitrate	68.2	87.2	105.3	86.9	
100	Urea	93.9	117.8	116.9	109.5	105.5
	Ammonium Nitrate	75.6	119.4	109.4	101.5	
Average		71.2	92.6	97.2		
Nitrogen treated average		77.9	101.9	106.3		

L.S.D. (.05) between temperatures - - - - - - - - - - - - - - - -11.12 mg.
L.S.D. (.01) between temperatures - - - - - - - - - - - - - - - 15.14 mg.
L.S.D. (.05) between rates - - - - - - - - - - - - - - - - - - 9.62 mg.
L.S.D. (.01) between rates - - - - - - - - - - - - - - - - - - 13.11 mg.

C.V. for entire experiment - - - - - - - - - - - - - - - - - - 13.47%
C.V. excluding checks - 13.79%

[a] Figures represent an average of three replications.

TABLE IX

MILLIGRAMS OF POTASSIUM PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Milligrams: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		69.9[a]	121.8	100.0	97.2	97.2
50	Urea	96.4	164.2	126.5	129.0	129.4
	Ammonium Nitrate	102.9	165.7	120.7	129.8	
100	Urea	106.4	189.4	132.3	142.7	144.9
	Ammonium Nitrate	97.4	213.1	131.0	147.1	
Average		94.6	170.8	122.1		
Nitrogen treated average		100.7	183.1	127.6		

L.S.D. (.05) between temperatures - - - - - - - - - - - - - - - - - 4.39 mg.

C.V. for entire experiment - 8.88%
C.V. excluding checks - 9.20%

[a] Figures represent an average of three replications.

TABLE X

MILLIGRAMS OF CALCIUM PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Milligrams: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		12.2[a]	22.2	26.4	20.3	20.3
50	Urea	16.8	34.8	35.1	28.9	28.6
	Ammonium Nitrate	18.2	29.1	37.5	28.3	
100	Urea	18.5	42.1	49.2	36.6	37.8
	Ammonium Nitrate	16.8	48.8	51.6	39.1	
Average		16.5	35.4	40.0		
Nitrogen treated average		17.6	38.7	43.4		

C.V. for entire experiment - 18.74%
C.V. excluding checks - 19.20%

[a] Figures represent an average of three replications.

TABLE XI

MILLIGRAMS OF MAGNESIUM PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Milligrams: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		7.6[a]	20.7	20.4	16.2	16.2
50	Urea	8.2	27.7	25.2	20.4	20.8
	Ammonium Nitrate	10.1	21.7	31.9	21.2	
100	Urea	10.8	31.9	27.8	23.5	25.4
	Ammonium Nitrate	9.2	40.9	32.0	27.4	
Average		9.2	28.6	27.5		
Nitrogen treated average		9.6	30.6	29.2		

L.S.D. (.05) between temperatures - - - - - - - - - - - - - - 15.85 mg.

C.V. for entire experiment - - - - - - - - - - - - - - - - - 13.76%
C.V. excluding checks - 13.99%

[a]Figures represent an average of three replications.

TABLE XII

MILLIGRAMS OF PHOSPHORUS PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

Pounds N/Acre	Source	Milligrams: Temperature 10°C	20°C	30°C	Avg.	Nitrogen Rate Avg.
0		3.9[a]	10.1	6.3	6.7	6.7
50	Urea	3.4	10.6	5.9	6.6	7.0
	Ammonium Nitrate	4.1	11.8	6.0	7.3	
100	Urea	5.4	10.6	7.2	7.7	7.4
	Ammonium Nitrate	4.0	10.8	6.1	7.0	
Average		4.2	10.8	6.3		
Nitrogen treated average		4.2	10.9	6.3		

L.S.D. (.05) between temperatures - - - - - - - - - - - - - - 1.2 mg.
L.S.D. (.01) between temperatures - - - - - - - - - - - - - - 1.6 mg.

C.V. for entire experiment - - - - - - - - - - - - - - - - - 19.65%
C.V. excluding checks - - - - - - - - - - - - - - - - - - - 19.95%

[a]Figures represent an average of three replications.

CHAPTER V

SUMMARY

The effects of temperature and urea concentration upon rate of urea hydrolysis and subsequent nitrification were determined. Yield and chemical composition of ryegrass forage as influenced by various soil temperatures and nitrogen treatments were also determined.

These determinations showed:

1. The conversion of urea to nitrate nitrogen was most rapid at the 30°C temperature and slowest at 10°C temperature.
2. Of the temperatures studied, 20°C appeared best suited for ryegrass growth.
3. The ryegrass responded to nitrogen fertilization up to the 100 pounds per acre rate, which was the highest rate used. The response was greatest at the 20°C temperature.
4. Urea and ammonium nitrate performed equally well as sources of nitrogen for ryegrass at all temperatures.
5. Nitrogen was more efficiently utilized by the ryegrass at the 20°C and 30°C temperatures than at the 10°C temperature.
6. The uptake of potassium, calcium, magnesium and phosphorus was retarded at the 10°C temperature.
7. Phosphorus uptake and yield of forage were directly related.
8. The calcium content of the ryegrass increased as the temperature and rate of nitrogen fertilization increased.

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LITERATURE CITED

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APPENDICES

APPENDIX A

VOLUMETRIC DETERMINATION OF AMMONIA NITROGEN IN SOILS

1. Place the equivalent of 100 grams air dry soil into 500 ml. erlenmeyer flask and add 250 ml. of acidified (pH 2.0) 10 per cent sodium chloride and shake for one hour.
2. Transfer to Buechner funnel, extract with an additional 250 ml. of the sodium chloride solution, retaining all of the leachate.
3. Place leachate into 800 ml. Kjeldahl flask and make alkaline with (10N) sodium hydroxide.
4. Distill over approximately 200 ml. into 50 ml. of 4 per cent boric acid containing modified methyl blue indicator.
5. Titrate using standard hydrochloric acid.
6. Calculate nitrogen content from amount of standard acid used.

APPENDIX B

DETERMINATION OF NITRATE NITROGEN COLORIMETRICALLY

1. Place the equivalent of 50 grams air dry soil into a 500 ml. flask.
2. Add 250 ml. copper and silver sulfate extract solution.
3. Place on shaker and shake for ten minutes.
4. Add 0.4 grams calcium hydroxide and 1.0 gram magnesium carbonate.
5. Shake for five minutes to precipitate the copper and silver.
6. Filter, discarding the first 20 ml. of the filtrate.
7. Set up a series of aliquots (0, 2, 5, 10 and 15 ml.) of the standard nitrate (10 p.p.m.) in three-inch porcelain evaporating dishes.
8. Measure a 10 ml. portion of the unknown filtrate into three-inch porcelain evaporating dishes.
9. Place all dishes on steam bath and evaporate to dryness in an atmosphere free of nitric acid fumes.
10. Allow to cool, and add 3 ml. of phenol-2-4disulphonic acid to the dishes, rotating the dishes to insure contact with all the salts.
11. Allow to stand ten minutes, then add 15 ml. of distilled water.
12. Make alkaline with ammonium hydroxide as indicated by an unchanging yellow color.
13. Transfer to 100 ml. volumetric flask and make up to volume with distilled water.
14. Read on spectrophotometer, using 410 mu. wave length, compare with standards, and calculate.

APPENDIX C

PROCEDURE FOR DETERMINATION OF pH OF SOILS

1. Place the equivalent of 25 grams air dry soil in 50 ml. beaker.
2. Add distilled water until thin pasty mixture is obtained.
3. Allow to set until soil becomes well saturated.
4. Re-mix and place electrodes of pH meter into the soil mixture and read pH directly.

APPENDIX D

PROCEDURE FOR DETERMINING THE NITROGEN CONTENT OF PLANT TISSUE

1. Place 1.0 gram oven dry (70°) plant tissue in Kjeldahl flask.
2. Add 10 grams catalyst (NA_2SO_4 and $CuSO_4 \cdot 5H_2O$ in ratio of 100:3.2) and 25 to 30 ml. of concentrated sulfuric acid.
3. Heat below boiling until frothing ceases, then raise temperature to the boiling point, and continue for thirty minutes after digestion is complete.
4. Cool; then add 200 ml. of distilled water.
5. Place 500 ml. erlenmeyer flask containing 50 ml. of 4 per cent boric acid containing modified methyl blue indicator beneath the delivery tube of the condenser.
6. Add sufficient (10N) sodium hydroxide to the digested material to make strongly alkaline; and add 4 to 5 pieces of mossy zinc.
7. Rapidly connect Kjeldahl flask to the condenser after addition of sodium hydroxide.
8. Distill over approximately 200 ml. into the boric acid.
9. Titrate the distillate using standard hydrochloric acid.
10. Calculate percentage nitrogen.

APPENDIX E

PROCEDURE FOR DETERMINATION OF POTASSIUM, CALCIUM, MAGNESIUM AND PHOSPHORUS CONTENT OF PLANT TISSUE

1. Weigh 0.5000 grams plant sample and transfer to a 150 ml. beaker, and cover with a watch glass.
2. Add 5 ml. of concentrated nitric acid and take to dryness on a hot plate at moderate heat.
3. Add 5 ml. of 1:1 nitric acid and 5 ml. of concentrated perchloric acid (70-72%) and take to dryness on a hot plate.
4. If ashing is complete, add 5 ml. dilute hydrochloric acid (1:1) and enough water to wash off the watch glass and warm gently.
5. Filter into a 250 ml. volumetric flask, using Whatman No. 42 filter paper, and make up to volume with distilled water.
6. Mix well and using small portions of the solution (5 ml.), determine the potassium, calcium and magnesium with a Beckman "DU" flame spectrophotometer equipped with a photomultiplier attachment. The wave lengths required are 766.5 mu. for potassium, 422.7 mu. for calcium and 285.2 mu. for magnesium.
7. The sample readings are compared with the calibration curve obtained from standard solutions and calculated.
8. For the phosphorus determination, place 2 ml. of the solution into a 25 ml. erlenmeyer flask and add 8 ml. of 0.05N hydrochloric acid, and 0.5 ml. of molybdic acid solution and mix.
9. Add 5 drops of reducing agent (amino-naphthol-sulfonic acid) and mix immediately.

10. Read in spectrophotometer at 650 mu. wave length 15 minutes after adding the reducing agent. Compare readings with those obtained for standard solutions and calculate phosphorus content of the unknown.

APPENDIX F

YIELD OF OVEN DRY RYEGRASS FORAGE IN GRAMS PER POT GROWN AT THREE TEMPERATURES WITH THREE RATES AND TWO SOURCES OF NITROGEN

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	3.05	2.41	2.62	2.69
2	50	Urea	"	3.35	3.17	2.50	3.01
3	100	"	"	4.05	4.02	3.20	3.76
4	50	NH_4NO_3	"	3.05	3.74	3.40	3.39
5	100	"	"	2.88	3.68	3.23	3.26
6	0		20°C	4.25	4.36	3.86	4.16
7	50	Urea	"	6.02	4.80	4.71	5.18
8	100	"	"	5.61	6.10	5.67	5.79
9	50	NH_4NO_3	"	5.21	4.95	4.80	4.99
10	100	"	"	6.10	6.20	5.98	6.09
11	0		30°C	3.25	3.55	3.35	3.38
12	50	Urea	"	3.72	3.58	4.30	3.87
13	100	"	"	5.05	4.00	4.35	4.47
14	50	NH_4NO_3	"	3.35	3.92	4.15	3.81
15	100	"	"	3.55	4.25	4.55	4.12

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	42.1516	3.0108	17.91**
N vs. No N	1	5.8284	5.8284	34.67**
Replications(a)	2	.1692	.0846	.50
Error(a)	28	4.7078	.1681	
Total(a)	44	47.0286		
Treatments(b)	11	33.1077	3.0980	15.60**
Temperature	2	29.0025	14.5012	73.02**
Rates	1	2.6406	2.6406	13.30**
Sources	1	.0407	.0407	.20
Temp. x Rates	2	.4931	.2466	1.24
Temp. x Sources	2	.7750	.3875	1.95
Rates x Sources	1	.1167	.1167	.59
Temp. x Rates x Sources	2	.0391	.0196	.10
Replications(b)	2	.1098	.0549	.27
Error(b)	22	4.3696	.1986	
Total(b)	35	37.5871		

**Significant at 0.01 level.

APPENDIX G

PERCENT OF NITROGEN IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	1.65	1.85	1.58	1.69
2	50	Urea	"	2.45	2.37	2.47	2.43
3	100	"	"	2.49	2.50	2.51	2.50
4	50	NH_4NO_3	"	2.50	2.02	1.56	2.03
5	100	"	"	2.80	2.52	1.66	2.33
6	0		20°C	1.38	1.28	1.16	1.27
7	50	Urea	"	1.93	1.35	1.59	1.62
8	100	"	"	2.14	1.90	2.07	2.04
9	50	NH_4NO_3	"	1.79	1.78	1.67	1.75
10	100	"	"	1.98	1.90	2.00	1.96
11	0		30°C	1.91	1.80	1.71	1.81
12	50	Urea	"	2.41	2.68	2.21	2.43
13	100	"	"	2.44	2.88	2.58	2.63
14	50	NH_4NO_3	"	2.87	2.77	2.68	2.77
15	100	"	"	2.40	2.83	2.70	2.64

ANALYSIS OF VARIANCE[a]

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	34.3433	2.4531	12.47**
N vs. No N	1	14.1905	14.1905	67.06**
Replications(a)	2	.9881	.4940	2.51
Error(a)	28	5.5086	.1967	
Total(a)	44	40.8400		
Treatments(b)	11	17.9662	1.6333	6.73**
Temperature	2	14.2339	7.1170	29.34**
Rates	1	1.0000	1.0000	4.12
Sources	1	.0177	.0177	.07
Temp. x Rates	2	.6093	.3046	1.26
Temp. x Sources	2	1.4217	.7108	2.93
Rates x Sources	1	.1273	.1273	.52
Temp. x Rates x Sources	2	.5563	.2782	1.15
Replications(b)	2	.8073	.4036	1.66
Error(b)	22	5.3378	.2426	
Total(b)	35	24.1113		

**Significant at 0.01 level.

a/ Figures represent degrees corresponding to percentages.

APPENDIX H

PERCENT OF POTASSIUM IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	2.40	2.95	2.50	2.62
2	50	Urea	"	3.05	3.05	1.50	2.53
3	100	"	"	2.50	3.05	2.60	2.72
4	50	NH_4NO_3	"	3.20	3.05	2.85	3.03
5	100	"	"	3.50	2.70	2.85	3.02
6	0		20°C	2.85	2.95	3.00	2.93
7	50	Urea	"	3.05	3.30	3.20	3.18
8	100	"	"	3.30	3.40	3.10	3.27
9	50	NH_4NO_3	"	3.65	2.90	3.40	3.32
10	100	"	"	3.40	3.35	3.75	3.50
11	0		30°C	3.20	2.60	3.10	2.97
12	50	Urea	"	3.30	3.45	3.10	3.28
13	100	"	"	2.90	3.00	3.00	2.97
14	50	NH_4NO_3	"	3.60	2.85	3.00	3.15
15	100	"	"	3.75	2.85	3.05	3.22

ANALYSIS OF VARIANCE[a]

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	9.2754	.6625	1.70NS
N vs. No N	1	1.2937	1.2937	3.31NS
Replications(a)	2	1.3513	.6756	1.73
Error(a)	28	10.9248	.3902	
Total(a)	44	21.5515		
Treatments(b)	11	7.2900	.6627	1.58
Temperature	2	4.5568	2.2784	5.42*
Rates	1	.0387	.0387	.09
Sources	1	1.2173	1.2173	2.89
Temp. x Rates	2	.3341	.1670	.40
Temp. x Sources	2	.6770	.3385	.80
Rates x Sources	1	.0225	.0225	.05
Temp. x Rates x Sources	2	.4436	.2218	.53
Replications(b)	2	1.8403	.9202	2.19
Error(b)	22	9.2510	.4205	
Total(b)	35	18.3813		

*Significant at 0.05 level.

[a] Figures represent degrees corresponding to percentages.

APPENDIX I

PERCENT OF CALCIUM IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replication 1	2	3	Average
1	0		10°C	0.50	0.45	0.40	0.45
2	50	Urea	"	0.50	1.10	0.35	0.65
3	100	"	"	0.45	0.45	0.60	0.50
4	50	NH_4NO_3	"	0.50	0.55	0.55	0.53
5	100	"	"	0.55	0.50	0.50	0.52
6	0		20°C	0.50	0.55	0.55	0.53
7	50	Urea	"	0.55	0.75	0.75	0.68
8	100	"	"	0.90	0.50	0.75	0.72
9	50	NH_4NO_3	"	0.55	0.60	0.60	0.58
10	100	"	"	0.75	0.90	0.75	0.80
11	0		30°C	0.90	0.70	0.75	0.78
12	50	Urea	"	1.00	1.00	0.75	0.92
13	100	"	"	1.15	1.15	1.00	1.10
14	50	NH_4NO_3	"	0.95	1.00	1.00	0.98
15	100	"	"	1.25	1.10	1.40	1.25

ANALYSIS OF VARIANCE[a]

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	26.1020	1.8644	7.63**
N vs. No N	1	2.6209	2.6209	10.73**
Replications(a)	2	.1596	.0798	.33
Error(a)	28	6.8389	.2442	
Total(a)	44	33.1005		
Treatments(b)	11	21.0747	1.9159	18.86**
Temperature	2	18.3011	9.1506	13.12
Rates	1	.7454	.7454	1.07
Sources	1	.0081	.0081	.08
Temp. x Rates	2	1.3946	.6973	6.86*
Temp. x Sources	2	.2348	.1174	1.16
Rates x Sources	1	.2916	.2916	2.87
Temp. x Rates x Sources	2	.0991	.0496	.49
Replications(b)	2	.2032	.1016	
Error(b)	22	6.4605		
Total(b)	35	27.7384		

*Significant at 0.05 level.
**Significant at 0.01 level.
a/Figures represent degrees corresponding to percentages.

APPENDIX J

PERCENT OF MAGNESIUM IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	0.30	0.30	0.25	0.28
2	50	Urea	"	0.30	0.30	0.20	0.27
3	100	"	"	0.30	0.30	0.25	0.28
4	50	NH_4NO_3	"	0.30	0.30	0.30	0.30
5	100	"	"	0.30	0.30	0.25	0.28
6	0		20°C	0.55	0.40	0.55	0.50
7	50	Urea	"	0.55	0.50	0.55	0.53
8	100	"	"	0.60	0.50	0.55	0.55
9	50	NH_4NO_3	"	0.50	0.40	0.40	0.43
10	100	"	"	0.60	0.70	0.71	0.67
11	0		30°C	0.71	0.50	0.61	0.61
12	50	Urea	"	0.71	0.71	0.55	0.66
13	100	"	"	0.71	0.71	0.72	0.71
14	50	NH_4NO_3	"	0.78	0.71	0.71	0.73
15	100	"	"	0.78	0.71	0.84	0.78

ANALYSIS OF VARIANCE[a]

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	24.6571	1.7612	36.02**
N vs. No N	1	.2471	.2471	5.05*
Replications(a)	2	.2141	.1070	2.19
Error(a)	28	1.3685	.0489	
Total(a)	44	26.2397		
Treatments(b)	11	21.2858	1.9351	48.99**
Temperature	2	19.7429	9.8714	40.06*
Rates	1	.4647	.4647	1.88
Sources	1	.0832	.0832	.34
Temp. x Rates	2	.3741	.1870	.76
Temp. x Sources	2	.0392	.0146	.05
Rates x Sources	1	.0889	.0889	.36
Temp. x Rates x Sources	2	.4928	.2464	6.24**
Replications(b)	2	.1040	.0520	1.32
Error(b)	22	.8685	.0395	
Total(b)	35	22.2583		

*Significant at 0.05 level.
**Significant at 0.01 level.
[a] Figures represent degrees corresponding to percentages.

APPENDIX K

PERCENT OF PHOSPHORUS IN OVEN DRY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	0.12	0.18	0.13	0.14
2	50	Urea	"	0.12	0.12	0.08	0.11
3	100	"	"	0.12	0.20	0.10	0.14
4	50	NH_4NO_3	"	0.12	0.12	0.12	0.12
5	100	"	"	0.13	0.12	0.12	0.12
6	0		20°C	0.19	0.26	0.29	0.25
7	50	Urea	"	0.21	0.22	0.18	0.20
8	100	"	"	0.18	0.19	0.18	0.18
9	50	NH_4NO_3	"	0.18	0.31	0.22	0.24
10	100	"	"	0.18	0.18	0.18	0.18
11	0		30°C	0.21	0.17	0.21	0.20
12	50	Urea	"	0.15	0.16	0.15	0.15
13	100	"	"	0.16	0.15	0.17	0.16
14	50	NH_4NO_3	"	0.16	0.14	0.18	0.16
15	100	"	"	0.18	0.14	0.14	0.15

ANALYSIS OF VARIANCE[a]

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	3.0665	.2190	12.44**
N vs. No N	1	.5868	.5868	33.34**
Replications(a)	2	.5372	.2686	15.26**
Error(a)	28	.4944	.0176	
Total(a)	44	4.0981		
Treatments(b)	11	2.2124	.2011	5.93**
Temperature	2	1.8889	.9444	27.86**
Rates	1	.0094	.0094	.003
Sources	1	.0075	.0075	.002
Temp. x Rates	2	.2151	.1076	3.17
Temp. x Sources	2	.0129	.0064	.002
Rates x Sources	1	.0711	.0711	2.10
Temp. x Rates x Sources	2	.0075	.0038	.001
Replications(b)	2	.1065	.0532	1.57
Error(b)	22	.7456	.0339	
Total(b)	35	3.0645		

**Significant at 0.01 level.

[a] Figures represent degrees corresponding to percentages.

APPENDIX L

MILLIGRAMS OF NITROGEN PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	50.3	44.6	41.4	45.4
2	50	Urea	"	82.1	75.1	61.8	73.0
3	100	"	"	100.8	100.5	80.3	93.9
4	50	NH_4NO_3	"	76.2	75.5	53.0	68.2
5	100	"	"	80.6	92.7	53.6	75.6
6	0		20°C	58.6	55.8	44.8	53.1
7	50	Urea	"	116.2	64.8	74.9	85.3
8	100	"	"	120.0	115.9	117.4	117.8
9	50	NH_4NO_3	"	93.2	88.1	80.2	87.2
10	100	"	"	120.8	117.8	119.6	119.4
11	0		30°C	62.1	63.9	57.3	61.1
12	50	Urea	"	89.6	95.9	95.0	93.5
13	100	"	"	123.2	115.2	112.2	116.9
14	50	NH_4NO_3	"	96.1	108.6	111.2	105.3
15	100	"	"	85.2	120.2	122.8	109.4

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	24134.38	1723.88	12.55**
N vs. No N.	1	12854.14	12854.14	93.61**
Replications(a)	2	646.43	323.22	2.35
Error(a)	28	3844.70	137.31	
Total(a)	44	28625.51		
Treatments(b)	11	10911.99	992.00	5.73**
Temperature	2	5772.88	2886.44	16.67**
Rates	1	3626.05	3626.05	20.94**
Sources	1	57.51	57.51	.33
Temp. x Rates	2	678.58	339.29	1.96
Temp. x Sources	2	362.51	181.26	1.05
Rates x Sources	1	271.69	271.69	1.57
Temp. x Rates x Sources	2	142.77	71.38	.41
Replications(b)	2	510.79	255.40	1.47
Error(b)	22	3809.99	173.18	
Total(b)	35	15232.77		

**Significant at 0.01 level.

APPENDIX M

MILLIGRAMS OF POTASSIUM PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	73.2	71.1	65.5	69.9
2	50	Urea	"	102.2	96.6	90.5	96.4
3	100	"	"	101.3	122.6	95.2	106.4
4	50	NH_4NO_3	"	97.6	114.1	96.9	102.9
5	100	"	"	100.8	99.4	92.1	97.4
6	0		20°C	121.1	128.6	115.8	121.8
7	50	Urea	"	183.6	158.4	150.7	164.2
8	100	"	"	185.1	207.4	175.8	189.4
9	50	NH_4NO_3	"	190.2	143.6	163.2	165.7
10	100	"	"	207.4	207.7	224.2	213.1
11	0		30°C	104.0	92.3	103.8	100.0
12	50	Urea	"	122.8	123.5	133.3	126.5
13	100	"	"	146.5	120.0	130.5	132.3
14	50	NH_4NO_3	"	126.0	111.7	124.5	120.7
15	100	"	"	133.1	121.1	138.8	131.0

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	63094.05	4506.72	34.14**
N vs. No N	1	11468.86	11468.86	86.87**
Replications(a)	2	334.50	167.25	1.27
Error(a)	28	3696.53	132.02	
Total(a)	44	67125.08		
Treatments(b)	11	47550.34	4322.76	27.14**
Temperature	2	42306.68	21153.34	21.22*
Rates	1	2171.56	2171.56	2.18
Sources	1	59.80	59.80	0.38
Temp. x Rates	2	1993.93	996.96	6.26**
Temp. x Sources	2	455.55	227.76	1.43
Rates x Sources	1	32.11	32.11	0.20
Temp. x Rates x Sources	2	530.71	265.36	1.67
Replications(b)	2	322.87	161.44	1.01
Error(b)	22	3504.01	159.27	
Total(b)	35	51377.22		

*Significant at 0.05 level.
**Significant at 0.01 level.

APPENDIX N

MILLIGRAMS OF CALCIUM PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	15.2	10.8	10.5	12.2
2	50	Urea	"	16.8	24.8	8.8	16.8
3	100	"	"	18.2	18.1	19.2	18.5
4	50	NH_4NO_3	"	15.2	20.6	18.7	18.2
5	100	"	"	15.8	18.4	16.2	16.8
6	0		20°C	21.3	24.0	21.2	22.2
7	50	Urea	"	33.1	36.0	35.3	34.8
8	100	"	"	53.3	30.5	42.5	42.1
9	50	NH_4NO_3	"	28.7	29.7	28.8	29.1
10	100	"	"	45.8	55.8	44.8	48.8
11	0		30°C	29.2	24.8	25.1	26.4
12	50	Urea	"	37.2	35.8	32.2	35.1
13	100	"	"	58.1	46.0	43.5	49.2
14	50	NH_4NO_3	"	31.8	39.2	41.5	37.5
15	100	"	"	44.4	46.8	63.7	51.6

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	7350.33	525.02	15.97**
N vs. No N	1	1211.09	1211.09	36.84**
Replications(a)	2	5.35	2.68	.08
Error(a)	28	920.56	32.87	
Total(a)	44	8276.24		
Treatments(b)	11	5819.96	529.09	13.03**
Temperature	2	4530.98	2265.49	12.12
Rates	1	773.77	773.77	4.14
Sources	1	7.56	7.56	.19
Temp. x Rates	2	373.76	186.88	4.60*
Temp. x Sources	2	10.99	5.49	.14
Rates x Sources	1	21.93	21.93	.54
Temp. x Rates x Sources	2	100.97	50.48	1.24
Replications(b)	2	1.76	.88	0.02
Error(b)	22	893.17	40.60	
Total(b)	35	6714.89		

*Significant at 0.05 level.
**Significant at 0.01 level.

APPENDIX O

MILLIGRAMS OF MAGNESIUM PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	9.2	7.2	6.5	7.6
2	50	Urea	"	10.1	9.5	5.0	8.2
3	100	"	"	12.2	12.1	8.0	10.8
4	50	NH_4NO_3	"	9.2	11.2	10.0	10.1
5	100	"	"	8.6	11.0	8.1	9.2
6	0		20°C	23.4	17.4	21.2	20.7
7	50	Urea	"	33.1	24.0	25.9	27.7
8	100	"	"	33.9	30.5	31.2	31.9
9	50	NH_4NO_3	"	26.0	19.8	19.2	21.7
10	100	"	"	36.9	43.4	42.4	40.9
11	0		30°C	23.1	17.8	20.4	20.4
12	50	Urea	"	26.4	25.4	23.7	25.2
13	100	"	"	35.8	28.4	31.5	31.9
14	50	NH_4NO_3	"	26.0	27.8	29.5	27.8
15	100	"	"	27.5	30.2	38.2	32.0

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	4672.18	333.73	37.33**
N vs. No N	1	338.67	338.67	37.88**
Replications(a)	2	24.69	12.34	1.38
Error(a)	28	250.35	8.94	
Total(a)	44	4947.22		
Treatments(b)	11	3999.75	363.61	34.82**
Temperature	2	3300.49	1650.24	20.25**
Rates	1	324.60	324.60	3.98
Sources	1	9.30	9.30	.11
Temp. x Rates	2	178.98	89.49	1.10
Temp. x Sources	2	3.05	1.52	.02
Rates x Sources	1	20.40	20.40	.25
Temp. x Rates x Sources	2	162.93	81.46	7.80**
Replications(b)	2	8.98	4.49	.43
Error(b)	22	229.66	10.44	
Total(b)	35	4238.39		

*Significant at 0.05 level.
**Significant at 0.01 level.

APPENDIX P

MILLIGRAMS OF PHOSPHORUS PER POT REMOVED BY RYEGRASS FORAGE GROWN WITH DIFFERENT NITROGEN FERTILIZATION AT THREE TEMPERATURES

No.	Pounds N/Acre	Source	Temperature	Replications 1	2	3	Average
1	0		10°C	3.81	4.34	3.46	3.87
2	50	Urea	"	4.19	3.96	2.00	3.38
3	100	"	"	4.94	8.04	3.20	5.39
4	50	NH_4NO_3	"	3.72	4.56	4.15	4.14
5	100	"	"	3.80	4.23	3.94	3.99
6	0		20°C	7.65	11.42	11.11	10.06
7	50	Urea	"	12.64	10.56	8.48	10.56
8	100	"	"	10.09	11.46	10.20	10.58
9	50	NH_4NO_3	"	9.38	15.44	10.60	11.81
10	100	"	"	10.68	11.16	10.50	10.78
11	0		30°C	6.89	5.96	5.96	6.27
12	50	Urea	"	5.58	5.80	6.45	5.94
13	100	"	"	8.18	6.00	7.31	7.16
14	50	NH_4NO_3	"	5.40	5.41	7.26	6.02
15	100	"	"	6.21	5.86	6.28	6.12

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sums of Squares	Means Square	F Value
Treatments(a)	14	354.84	25.34	13.13**
N vs. No N	1	1.29	1.29	.67
Replications(a)	2	6.75	3.38	1.75
Error(a)	28	54.15	1.93	
Total(a)	44	415.74		
Treatments(b)	11	295.11	26.83	13.15**
Temperature	2	282.61	141.30	69.26**
Rates	1	1.17	1.17	.57
Sources	1	.01	.01	.005
Temp. x Rates	2	3.47	1.74	.85
Temp. x Sources	2	2.99	1.50	.74
Rates x Sources	1	4.71	4.71	2.31
Temp. x Rates x Sources	2	.15	.08	.04
Replications(b)	2	6.25	3.12	1.53
Error(b)	22	44.92	2.04	
Total(b)	35	346.28		

**Significant at 0.01 level.